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# The effect of natural extracts and tea compost in some root growth parameters of Calendula plant under stress by heavy elements

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## Abstract

A factorial experiment was carried out in one of the greenhouses to study the ability of the calendula plants to accumulate and resist growth in soil contaminated with toxic elements and plant treatment using plant extracts and treatment with different proportions of bio-organic aqueous extracts in some root growth indicators. The experiment included the use of three factors. The first factor included two types of plant extracts (licorice root and malt grain) in addition to the control treatment, while the second factor was treating the planting soil with two ratios of tea compost (1/5 and 1/10). The third factor was the treatment of Potting soil with the heavy elements copper and lead and their mixture at a concentration of 5 mg $kg^{-1}$, the experiment applied according R.C.D with three replicate, and each replicate included 3 experimental units. The results of the study showed a significant impact in the characteristics of the root growth. The intervention treatment (malt extract and a 1/5 ratio of tea compost for calendula plants stressed with two elements lead and copper and those not stressed) had a significant advantage in root length and the number of roots with an average of 39.33 cm and 16.67 roots, respectively. While the interaction treatment (licorice extract not treated with tea compost and stressed with copper) achieved an average of 3.292 gm for the dry weight of the root system, the two triple interaction treatments (malt extract, not treated with tea compost and stressed with lead, and treated with licorice extract, not treated with tea compost and stressed with copper) achieved an average of (44.66, 16.320) mg kg $soil^{-1}$ root content of lead and copper.

**Keywords**: Tea compost, licorice root extract, Malt grain extract, heavy elements

## Introduction

Calendula (*Calendula officinalis* L.) is a winter herbaceous plant belonging to the Asteraceae family, It is native to the Mediterranean basin and is considered a fast-growing, early-flowering, and wonderfully beautiful ornamental plant. In addition to its harmonizing value, calendula is considered a medicinal plant, as it is a source of effective compounds that have anti-inflammatory and anti-mutagenic effects; it can also be used to treat diseases of the digestive system and gynecology. It can also treat skin injuries using Calendula flower petals [1, 2].

In their external surroundings, plants are exposed to various biotic and non-biotic stresses, the harmful effects of which appear on the plant. The stress force is often measured by the survival of the plant, the abundance of the crop, or the processes of fixing $CO_2$ and taking up the elements with which plant growth is linked , The stress of heavy elements is considered one of the stresses, Abiotic stresses, which contain highly toxic elements. Heavy elements have received great attention in the past few years as a result of the increasing manifestations of environmental pollution due to agriculture, industry, energy, local waste, and wastewater [3,4]. This is because they can cause damage when transferred to living organisms. What raises concern when environmental pollution with heavy elements occurs is that these metals are not biologically degradable, but rather have the ability to transform from one oxidized form or from one organic complex to another. The problem of environmental pollution has recently been raised, and there are real concerns about heavy metals as a result of their transfer to edible plants through their accumulation in their tissues at toxic levels, as well as the animals consuming these plants as a result of the toxicity of these metals and the flow of their movement in the soil and plants, causing disturbances and physiological damage inside the plant. These disturbances are manifested by the effect on plastid membranes, chlorophyll, the work of enzymes, and the absorption of elements. The use of extracts is the natural properties of some plants used in organic agriculture that stimulate vegetative growth and improve the production of many crops. The effectiveness of plant extracts of medicinal and aromatic plants is due to them containing active substances that affect the growth of microbes and pathogenic bacteria. These active compounds result from photosynthesis, including tannins, glycosides, terpenes, and other active substances 2010). The current study aims to study the root growth indicators of calendula plants growing in contaminated soil and to study the ability of their roots to biologically process heavy elements using the principles of clean agriculture, which include natural extracts and bioorganic mixtures.

## Materials and Methods

A factorial experiment was carried out in a plastic house designed for this purpose for the period from June 2022 to March 2023, to study the ability of the calendula plant to accumulate and resist growth in soil contaminated with toxic elements and plant treatment using natural extracts from different plant parts and treatment with different ratios of bio-organic aqueous extracts. The seeds of the plant were planted on September 26, 2022, in the form of seedlings, through cork dishes (eyes) filled with peat moss medium only. When they reached the seedling stage with the appearance of two pairs of true leaves, they were placed in 8 kg plastic planting pots filled with peat moss medium + River soil) at a ratio of 1:3, one plant per pot, to implement the study factors under greenhouse conditions. The experiment was carried out using a completely randomized design (C.R.D.), with three replications and three factors as follows.

**The first factor**: Two types of natural plant extracts were used depending on their plant source, in addition to the control treatment as foliar spray treatments, with three spraying periods and a time difference of 15 days between each spray and the next, starting from the first spray, as follows: extract of licorice roots and barley grains at a concentration of 100% ml $L^{-1}$. The Licorice extract was prepared after plant collection was completed using the aqueous extraction method according to the method described by [5].

**The Second factor**: two ratios of tea compost were used in addition to the control treatment as a ground additive with three addition periods (Tea Compost ratio means the amount of fermented organic matter to the volume of water in a 100-liter container) as follows: the treatment with water only and treatment in the ratio of 1/5 and 1/10. Decomposed organic materials of plant origin were collected from organic farms on June 25, 2022. They were cleaned and then packed under a black polyethylene cover for 75 days to ensure their complete decomposition, with continuous stirring and detection; and quantities of industrial molasses were added every 15 days through the accumulation period. Plastic barrels of 120 liters were then prepared and filled with twice-distilled, chlorine-free water, according to the proportions shown above. The planting pots were treated by adding the above extract according to the prepared proportions to the soil three days before the separation process was carried out to ensure the homogeneity of the soil with the solution, only once.

**The Third factor**: The agricultural soil was contaminated with heavy elements before starting to spread the seedlings as follows: planting in naturally mixed soil, adding lead and copper and their mixture at a concentration of 5 mg $L^{-1}$. The heavy metals were added in the form of solutions according to the proven concentrations in equal quantities to the planting pots after dissolving them in 1 liter of water, then they were added in one batch, mixing with the soil. This process took place 15 days before planting the seedlings to ensure a homogeneous distribution of the elements in the potting soil.

**The Parameters**

1- **Root length (cm):** This characteristic was measured after the end of the research experiment, as the plants were removed from the pot after randomly selecting one plant from each replicate of each treatment, and then washing it well with water to remove dust; the measurement was done with a metric tape, starting from the crown area to the terminal end for the root.
2- **Number of roots (plant $root^{-1}$):** The number of branch roots of one plant from each replicate was calculated.
3- **Dry weight of the root system (gm):** One plant was selected from each replicate and after cleaning and washed well with water, after which it was dried in

an electric oven at a temperature of 70°C until the weight was stable, and then the dry weight was measured using a balance sensitive.

4- **The roots contain heavy elements lead and copper (mg kg soil$^{-1}$):** the plant parts were washed with ion-free distilled water and dried at a temperature of (70) °C, then ground and passed through a 40-mesh sieve, the dried and ground plant samples were digested with the acid mixture. ($HClO_4$: $H_2SO_4$ (sulfuric: perchloric) by adding 5 ml of sulfuric acid to 0.2 gm of plant sample for 24 hours and then placing it at a temperature of 80°C for an hour on a heat plate; then it cooled airily for a period of time, then we added 3 ml of perchloric acid ($HClO_4$) for a period of time on a hot plate until the colour turns from dark brown to clear, colourless. Then the samples were filtered with what man No.42 filter paper, and then the volume was increased to 50 ml. After that, the concentrations of heavy metals (lead and copper) were measured using an atomic absorption spectrophotometer, which was calibrated with standard solutions of lead and copper, and the result was expressed in mg kg$^{-1}$, according to the method [6].

## Results and Discussion

### First/ Root length (cm):

Treating the calendula plant with different types of natural extracts caused a significant difference in root length, as shown by the results of Table (1), as the treatment with the licorice extract excelled in showing the highest results for the root stage of the plant, achieving an average of 30.56 cm, thus outperforming the rest of the two treatments in control; with the treatment of spraying with malt extract, the lowest average was recorded 28.28 cm. Also significant differences were found in the same trait due to the treatment of plants with tea compost, the control treatment outperformed and recorded the highest average of 31.06 cm, while the lowest averages were recorded when treating the 1/5 ratio of calendula plants with an average of 28.50cm. The same table also shows that abiotic stress has a significant effect on the same trait. The treatment of the mixture with heavy elements achieved the highest average root length of 33.30 cm, thus significantly outperforming the other treatments, while the control treatment recorded the lowest average for this trait of 25.30 cm. As for the binary interaction between spraying natural extracts and adding tea compost, the results of the table showed that there was a significant difference in the control treatment, which achieved the highest average of 33.58cm. In comparison, the interaction of not spraying calendula plants with the natural extract and adding the ratio of 1/5 achieved the lowest average of 26.50 cm. The case was not different when spraying the natural extract was mixed with heavy elements, as the treatment (licorice extract with stress with both elements) recorded the highest average length of the roots of plants 35.11cm, while the interaction (spraying with malt extract of unstressed plants) gave the lowest average of 24.56. The interaction of adding tea compost to pots of plants stressed with heavy elements had a significant effect on root length, as the treatment of plants not treated with tea compost and stressed with the mixture of heavy elements recorded an average of 37.11cm, compared to the interaction of 1/10 for plants not stressed with heavy elements, as the lowest average it reached was 24.00 cm. According to the results shown in the same Table, the triple interaction had a significant effect on root length, as treating calendula plants with licorice extract and not adding tea compost stressed with a mixture of heavy elements recorded the highest average of (39.33 cm), while the triple interaction treatment by spraying the plants with malt extract recorded those not treated with tea compost and not stressed with heavy elements had the lowest average of 19.67 cm.

**Table (1): Effect of natural extract and tea compost and their interaction on the root length of calendula plants (cm) stressed by heavy elements**

| *Extract* | *Tea compost* | *Heavy Elements* | | | | *Extract × Heavy Elements ×Tea compost* |
|---|---|---|---|---|---|---|
| | | *Non* | *Cu* | *Pb* | *Pb + Cu* | |
| *Non* | *Non* | 29.33 | 32.33 | 33.67 | 39.00 | 33.58 |
| | **1/5** | 24.67 | 26.00 | 24.67 | 30.67 | 26.50 |
| | **1/10** | 21.00 | 29.00 | 30.33 | 30.33 | 27.67 |
| *Malt* | *Non* | 19.67 | 27.67 | 29.67 | 33.00 | 27.50 |
| | **1/5** | 28.33 | 29.67 | 27.33 | 32.33 | 29.42 |
| | **1/10** | 25.67 | 29.33 | 27.67 | 29.00 | 27.92 |
| *Licorice* | *Non* | 26.33 | 32.67 | 30.00 | 39.33 | 32.08 |
| | **1/5** | 27.33 | 30.67 | 27.00 | 33.33 | 29.58 |
| | **1/10** | 25.33 | 33.00 | 29.00 | 32.67 | 30.00 |
| L.S.D. 0.05 | 4.226 | | | | | 2.133 |
| *Extract × Heavy Elements* | | | | | | *Extract* |
| *Non* | | 25.00 | 29.11 | 29.56 | 33.33 | 29.25 |
| *Malt* | | 24.56 | 28.89 | 28.22 | 31.44 | 28.28 |
| *Licorice* | | 26.33 | 32.11 | 28.67 | 35.11 | 30.56 |
| **L.S.D. 0.05** | | 2.440 | | | | 1.220 |
| *Tea compost × Heavy Elements* | | | | | | *Tea compost* |
| *Non* | | 25.11 | 30.89 | 31.11 | 37.11 | 31.06 |
| **1/5** | | 26.78 | 28.78 | 26.33 | 32.11 | 28.50 |
| **1/10** | | 24.00 | 30.44 | 29.00 | 30.67 | 28.53 |
| L.S.D. 0.05 | | 2.440 | | | | 1.220 |
| *Heavy Elements* | | 25.30 | 30.04 | 28.81 | 33.30 | |

| | |
|---|---|
| L.S.D. 0.05 | 1.409 |

**Second/ Number of roots (plant root$^{-1}$):**

Table (2) shows the presence of significant differences in the character of the number of roots of calendula plants due to the effect of treating the plants with different types of natural extracts sprayed on the shoots. The control treatment was significantly superior to all treatments for plants by achieving the highest average for this characteristic, amounting to 10.94 roots, while the lowest average when treating plants with licorice extract was 10.03 roots. As for the effect of tea compost, the results of the Table showed that there were significant differences in the above characteristic resulting from treating the plants with the 1/5 ratio, recording the highest average of 13.17 roots, compared to the lowest average that was achieved when treating the 1/10 ratio with an average of 7.39 roots for the calendula plant. The Table also shows that abiotic stress has a significant effect on the same trait, the control treatment achieved the highest average for the number of roots, with an average of 12.30 roots, thus significantly outperforming the other treatments, while the stress treatment with a mixture of heavy elements recorded the lowest average for this trait, with an average of 7.59 roots.

According to the results presented in the same table, the binary interactions between the experimental factors caused a significant difference in the number of plant roots. The interaction of spraying with natural extracts and adding tea compost to the potting soil achieved a significant response, represented by the interaction treatment of spraying calendula plants with malt extract and the ratio of 1/5 achieved the highest average 14.58 roots, while the lowest average was achieved when plants were treated with a ratio of 1/10 and not treated with natural extracts, as the lowest average was 7.00 roots. As for the interaction of spraying plant extracts and stress, the interaction of spraying plants with licorice extract that were not stressed caused a significant increase in the number of roots with the highest average of 12.56 roots, in contrast to the treatment of spraying plants with malt extract and stressed with a mixture of heavy elements, which gave the lowest average of 7.00 roots. The results of the same Table indicated that there were significant differences caused by the interaction of tea compost with heavy elements, as the treatment of overlapping the ratio of 1/5 that was not stressed with heavy elements recorded a significant superiority over the rest of the treatments by achieving the highest average of 15.33 roots, compared to the interaction of adding the ratio of 1/10 to the stressed plants, with a mixture of heavy elements that recorded the lowest average of 6.00 roots. The triple interaction between the study factors caused a significant effect on the number of roots, as the interaction of spraying calendula plants with malt extract and the 1/5 ratio stressed with copper recorded the highest average of 17.00 roots. In contrast, the lowest average number of roots was achieved when treated by spraying plants with malt extract without treatment, with the biological organic mixture and strained with the mixture of heavy elements, with an average of 4.33 roots for the same trait.

**Table (2) :The effect of natural extract and tea compost and their interaction on the number of roots of calendula plants stressed by heavy elements**

| *Extract* | *Tea compost* | *Heavy Elements* | | | | *Extract × Heavy Elements* |
|---|---|---|---|---|---|---|
| | | *Non* | *Cu* | *Pb* | *Pb + Cu* | |
| *Non* | *Non* | 12.67 | 14.33 | 14.00 | 10.33 | 12.83 |
| | **1/5** | 15.67 | 14.33 | 14.33 | 7.67 | 13.00 |
| | **1/10** | 7.67 | 7.00 | 6.33 | 7.00 | 7.00 |
| *Malt* | *Non* | 11.67 | 7.33 | 11.33 | 4.33 | 8.67 |
| | **1/5** | 16.67 | 17.00 | 13.33 | 11.33 | 14.58 |
| | **1/10** | 8.67 | 8.00 | 6.67 | 5.33 | 7.17 |
| *Licorice* | *Non* | 12.67 | 11.67 | 8.33 | 8.00 | 10.17 |
| | **1/5** | 13.67 | 12.33 | 13.00 | 8.67 | 11.92 |
| | **1/10** | 11.33 | 8.67 | 6.33 | 5.67 | 8.00 |
| L.S.D. 0.05 | 2.328 | | | | | 1.164 |
| *Extract × Heavy Elements* | | | | | | *Extract* |
| *Non* | | 12.00 | 11.89 | 11.56 | 8.33 | 10.94 |
| *Malt* | | 12.33 | 10.78 | 10.44 | 7.00 | 10.14 |
| *Licorice* | | 12.56 | 10.89 | 9.22 | 7.44 | 10.03 |
| **L.S.D. 0.05** | | 1.344 | | | | 0.672 |
| *Tea compost × Heavy Elements* | | | | | | *Tea compost* |
| *Non* | | 12.33 | 11.11 | 11.22 | 7.56 | 10.56 |
| **1/5** | | 15.33 | 14.56 | 13.56 | 9.22 | 13.17 |
| **1/10** | | 9.22 | 7.89 | 6.44 | 6.00 | 7.39 |
| L.S.D. 0.05 | | 1.344 | | | | 0.672 |
| *Heavy Elements* | | 12.30 | 11.19 | 10.41 | 7.59 | |
| L.S.D. 0.05 | | 0.776 | | | | |

**Third/ Dry weight of the root system (gm):**

The statistical analysis data in Table (3) indicate that there are significant differences in the dry weight of the root system of calendula plants as a result of treating the plants with natural extracts, as the treatment of spraying plants with malt extract was significantly superior to the control treatment only, without differing significantly with the treatment of spraying with licorice extract, with both achieving a higher average that amounted to 1.851gm, while the control treatment recorded the lowest average for plants, 1.315 gm. As for the effect of tea compost, the results of the same table showed that there were significant differences in the aforementioned characteristic, as the control treatment achieved a significant superiority over the other treatments by recording the highest average of 1.863 gm, compared to the 1/10 ratio treatment, which recorded the lowest average of 1.456 gm. The results of the same

Table showed that abiotic stress has a significant effect on the same trait, the control treatment achieved a significant effect on all treatments, with the highest average dry weight of the root system, 1.929 gm, thus being significantly superior to the other treatments, while the stress treatment with lead only recorded the lowest average of 1.472 gm.

**Table (3): The effect of natural extract and tea compost and their interaction on the dry weight of the root system of calendula plants (gm) stressed by heavy elements**

| *Extract* | *Tea compost* | *Heavy Elements* | | | | *Extract × Heavy Elements* |
|---|---|---|---|---|---|---|
| | | *Non* | *Cu* | *Pb* | *Pb + Cu* | |
| *Non* | *Non* | 1.698 | 1.848 | 1.075 | 1.271 | 1.473 |
| | **1/5** | 1.334 | 1.480 | 1.219 | 1.420 | 1.363 |
| | **1/10** | 1.187 | 1.099 | 1.084 | 1.065 | 1.10 |
| *Malt* | *Non* | 2.494 | 1.727 | 1.855 | 1.583 | 1.915 |
| | **1/5** | 1.944 | 1.956 | 1.763 | 1.647 | 1.827 |
| | **1/10** | 1.947 | 1.910 | 1.757 | 1.624 | 1.810 |
| *Licorice* | *Non* | 3.292 | 2.108 | 1.764 | 1.643 | 2.202 |
| | **1/5** | 1.680 | 1.417 | 1.426 | 2.355 | 1.719 |
| | **1/10** | 1.784 | 1.518 | 1.308 | 1.188 | 1.450 |
| L.S.D. 0.05 | 0.313 | | | | | 0.157 |
| *Extract × Heavy Elements* | | | | | | *Extract* |
| *Non* | | 1.406 | 1.476 | 1.126 | 1.252 | 1.315 |
| *Malt* | | 2.129 | 1.864 | 1.792 | 1.618 | 1.851 |
| *Licorice* | | 2.252 | 1.681 | 1.499 | 1.729 | 1.790 |
| **L.S.D. 0.05** | | 0.181 | | | | 0.090 |
| *Tea compost × Heavy Elements* | | | | | | *Tea compost* |
| *Non* | | 2.495 | 1.894 | 1.564 | 1.499 | 1.863 |
| **1/5** | | 1.653 | 1.618 | 1.469 | 1.807 | 1.637 |
| **1/10** | | 1.639 | 1.509 | 1.383 | 1.292 | 1.456 |
| L.S.D. 0.05 | | 0.181 | | | | 0.090 |
| *Heavy Elements* | | 1.929 | 1.674 | 1.472 | 1.533 | |
| L.S.D. 0.05 | | 0.104 | | | | |

According to what was stated in the results of the Table, the binary interactions had a significant difference in this characteristic, as the interaction of spraying with natural extracts and adding tea compost to the potting soil achieved a significant response, represented by the interaction of licorice extract with the treatment without adding tea compost, with a higher average of 2.202 gm while the lowest average was achieved when spraying with water only and the ratio was 1/10, as the lowest average reached 1.109 gm. As for the interaction of plant extracts and stress treatments, the interaction of the licorice extract treatment of non-stressed plants resulted in a significant increase in this trait with the highest average of 2.252 gm, in contrast to the treatment of not spraying the plants with the natural extract and the plants stressed with lead, which recorded the lowest average of 1.126 gm for the same trait. In the same context, the results of the Table showed that there were significant differences caused by the interaction of adding tea compost to the pots of plants stressed with heavy elements, as the control treatment recorded a significant superiority over the rest of the treatments with an average of 2.495 gm, compared to the interaction of the ratio of 1/10 for plants stressed with the mixture of heavy elements, which achieved the lowest average of 1.292 gm. The triple interaction produced significant differences in this trait, as the treatment of spraying plants with licorice extract and not adding tea compost that was not stressed with heavy elements recorded the highest average of 3.292 gm, compared to 1.065 gm which was achieved when treating plants with a mixture of heavy elements and the ratio was 1/10 treatment with natural extracts.

**Forth/ Lead content of Plant roots (mg kg soil$^{-1}$):**

The statistical analysis data presented in Table (4) indicate that there are significant differences in the above-mentioned trait of the calendula plant due to the effect of treating the plants with different types of natural extracts by spraying on the shoots. The spraying treatment with the malt extract was significantly superior to all treatments, with the two achieving the highest average for this trait of 7.158. mg, while the lowest average was recorded when spraying with licorice extract, an average of 2.165 mg. As for the effect of tea compost, the results of the same table showed that there were significant differences resulting from treating plants with different ratios of the organic-bio mixture; the 1/5 ratio treatment achieved a significant effect on the other treatments by recording the highest average of 5.064 mg, while the control treatment achieved the lowest average of 5.064 mg. 3.319 mg. Table (4) indicates that the abiotic stress represented by heavy elements has a significant effect on the same trait. The stress treatment with lead only achieved a significant effect on all treatments, with the highest average reaching 8.520 mg, while the control treatment recorded the lowest average for this trait, amounting to 0.504 mg.

According to the results presented in the same table, the binary interactions between the experimental factors caused a significant difference in the plant’s height. The interaction of spraying with natural extracts and adding tea compost to the potting soil achieved a significant response, represented by the interaction of the malt extract treatment with the 1/5 ratio treatment, which recorded the highest average of 9.735 mg, while the lowest average was achieved when treating plants by spraying with water only and not adding the organic-biological mixture, an average of 0.024 mg. As for the interaction between spraying plant extracts and stress treatments, it had a significant effect, achieving 13.516 mg of the interaction between malt extract for plants stressed with lead only, in contrast to the treatment of spraying plants with licorice extract and not stressed with heavy elements, which gave the lowest average of 0.000 mg. The results of the same table showed that there were significant differences caused by the interaction of adding tea compost to the pots of plants stressed with heavy elements. The 1/5 interaction treatment for plants stressed with lead recorded a significant superiority over the rest of the treatments by achieving the highest average of 9.595 mg, compared to the interaction of the treatment of not adding the biological mixture organic for non-stressed plants, which recorded the lowest average of 0.038 mg. The interaction of the three study factors combined (natural extracts, tea compost, and abiotic stress) had a significant effect on the lead content of the roots. Treating the plants by spraying with malt extract and not adding tea compost stressed with heavy lead elements only recorded the highest average of (16.320 mg), compared to (0.000 mg), which was achieved when treating plants with several treatments (treatment of overlapping plants not treated with natural extracts, not adding the organic-biological mixture, and not stressed with heavy elements, and treatment by spraying licorice extract on plants not subjected to tea compost, and treatment in the ratio of 1/5 and the ratio of 1/10 for plants not stressed by elements heavy).

**Table (4) :The effect of natural extract and tea compost and their interaction on the lead content of the roots of calendula plants (mg kg soil$^{-1}$) stressed by heavy elements**

| *Extract* | *Tea compost* | *Heavy Elements* | | | | *Extract × Heavy Elements* |
|---|---|---|---|---|---|---|
| | | *Non* | *Cu* | *Pb* | *Pb + Cu* | |
| ***Non*** | ***Non*** | 0.000 | 0.017 | 0.035 | 0.041 | 0.024 |
| | **1/5** | 0.001 | 3.632 | 11.369 | 5.094 | 5.024 |
| | **1/10** | 0.001 | 0.069 | 8.495 | 6.608 | 3.793 |
| ***Malt*** | ***Non*** | 0.113 | 2.460 | 16.320 | 7.952 | 6.711 |
| | **1/5** | 1.914 | 8.004 | 15.762 | 13.259 | 9.735 |
| | **1/10** | 2.506 | 2.360 | 8.466 | 6.780 | 5.028 |
| ***Licorice*** | ***Non*** | 0.000 | 1.713 | 6.639 | 4.532 | 3.221 |
| | **1/5** | 0.000 | 0.011 | 1.653 | 0.063 | 0.432 |
| | **1/10** | 0.000 | 0.079 | 7.943 | 3.339 | 2.841 |
| L.S.D. 0.05 | 1.177 | | | | | 0.588 |
| *Extract × Heavy Elements* | | | | | | *Extract* |
| ***Non*** | | 0.000 | 1.239 | 6.633 | 3.914 | 2.947 |
| ***Malt*** | | 1.511 | 4.275 | 13.516 | 9.331 | 7.158 |
| ***Licorice*** | | 0.000 | 0.601 | 5.412 | 2.645 | 2.165 |
| **L.S.D. 0.05** | | 0.679 | | | | 0.340 |
| *Tea compost × Heavy Elements* | | | | | | *Tea compost* |
| ***Non*** | | 0.038 | 1.397 | 7.665 | 4.175 | 3.319 |
| **1/5** | | 0.638 | 3.882 | 9.595 | 6.139 | 5.064 |
| **1/10** | | 0.836 | 0.836 | 8.301 | 5.576 | 3.887 |

| | | | | | |
|---|---|---|---|---|---|
| L.S.D. 0.05 | 0.679 | | | | 0.340 |
| ***Heavy Elements*** | 0.504 | 2.038 | 8.520 | 5.297 | |
| L.S.D. 0.05 | 0.392 | | | | |

**Fifth/ Copper content of Plant roots (mg kg soil$^{-1}$):**

The statistical analysis data presented in Table (5) indicate that there are significant differences in the above-mentioned trait of the calendula plant due to the effect of treating the plants with different types of natural extracts by spraying them on the shoots. The spraying treatment with licorice extract was significantly superior to all treatments by achieving the highest average for this trait of 10.21 mg, while the lowest average was recorded in the control treatment of 6.44 mg. As for the effect of Tea compost, the results of the same table showed that there were significant differences in the above-mentioned characteristic resulting from treating plants with different ratios of the organic-bio mixture, as the control treatment achieved a significant effect on the other treatments by recording the highest average of 8.98 mg, while the treatment achieved the ratio of 1/10 The lowest average was 6.99 mg. The results of the same table also showed that abiotic stress represented by heavy elements has a significant effect on the same trait; the stress treatment with copper only achieved a significant effect on all treatments, with the highest average reaching 16.30 mg, while the control treatment recorded the lowest average for this trait 1.06 mg.

According to the results presented in the same table, the binary interactions between the experimental factors caused a significant difference in the character of this trait. The interaction of spraying with natural extracts with the addition of tea compost to the potting soil achieved a significant response, represented by the interaction treatment of licorice extract without adding the organic-bio mixture, with the highest average recorded at 16.71 mg, while the lowest average was achieved when treating plants by spraying with water only and not adding the organic-biological mixture; the lowest average was 4.98 mg. As for the interaction between spraying plant extracts and stress treatments, it had a significant effect, reaching 15.22 mg. A significant increase in the copper content of the roots was achieved from the treatment of not spraying plant extracts of plants stressed with copper only, while the interaction treatment of untreated plants and spraying treatment with malt extract of non-stressed plants recorded a significant increase in copper content in the roots, stressed with heavy elements the minimum average capacity is 0.00. The results of the same table showed that there were significant differences caused by the interaction of adding tea compost to pots of plants stressed with heavy elements, as the interaction treatment of not adding the organic-biological mixture to plants stressed with copper only recorded a significant superiority over the rest of the treatments by achieving the highest average of 21.59 mg, compared to the interaction of the ratio treatment 1/10 for plants not stressed with heavy metals, which recorded the lowest average of 0.45 mg. The interaction of the three study factors combined (natural extracts, tea compost, and abiotic stress) had a significant effect on the copper content of the roots, as treating plants sprayed with licorice extract and not adding tea compost stressed with heavy metals, copper only, recorded the highest average of 44.66 mg, compared to 0.00 which was achieved when treating plants with several treatments (treatment of overlapping untreated plants with natural extracts, not adding the organic-biological mixture, and not stressed with heavy elements, spraying with water only at a ratio of 1/5 for non-stressed plants, and spraying with malt extract at a ratio of 1/5 and a ratio of 1/10 For plants not stressed by heavy elements).

**Table (5): The effect of natural extract and tea compost and their interaction on the copper content of the roots of calendula plants (mg kg soil$^{-1}$) stressed with heavy elements**

| *Extract* | *Tea compost* | *Heavy Elements* | | | | *Extract × Heavy Elements* |
|---|---|---|---|---|---|---|
| | | *Non* | *Cu* | *Pb* | *Pb + Cu* | |
| *Non* | *Non* | 0.00 | 11.43 | 1.90 | 6.58 | 4.98 |
| | **1/5** | 0.00 | 15.20 | 6.34 | 8.86 | 7.60 |
| | **1/10** | 0.01 | 19.04 | 2.46 | 5.41 | 6.73 |
| *Malt* | *Non* | 0.01 | 8.68 | 5.03 | 7.34 | 5.26 |
| | **1/5** | 0.00 | 11.63 | 7.69 | 9.37 | 7.17 |
| | **1/10** | 0.00 | 11.60 | 7.72 | 9.13 | 7.11 |
| *Licorice* | *Non* | 6.18 | 44.66 | 5.33 | 10.67 | 16.71 |
| | **1/5** | 2.00 | 14.03 | 1.37 | 9.77 | 6.79 |
| | **1/10** | 1.34 | 10.46 | 7.36 | 9.33 | 7.12 |
| L.S.D. 0.05 | 1.899 | | | | | 0.950 |
| *Extract × Heavy Elements* | | | | | | *Extract* |
| *Non* | | 0.00 | 15.22 | 3.57 | 6.95 | 6.44 |
| *Malt* | | 0.00 | 10.63 | 6.81 | 8.61 | 6.52 |
| *Licorice* | | 3.17 | 23.05 | 4.69 | 9.92 | 10.21 |
| **L.S.D. 0.05** | | 1.097 | | | | 0.548 |
| *Tea compost × Heavy Elements* | | | | | | *Tea compost* |
| *Non* | | 2.06 | 21.59 | 4.09 | 8.20 | 8.98 |
| **1/5** | | 0.67 | 13.62 | 5.13 | 9.33 | 7.19 |
| **1/10** | | 0.45 | 13.70 | 5.85 | 7.96 | 6.99 |
| L.S.D. 0.05 | | 1.097 | | | | 0.548 |
| *Heavy Elements* | | 1.06 | 16.30 | 5.02 | 8.50 | |
| L.S.D. 0.05 | | 0.633 | | | | |

The difference in values for the studied traits shown in Tables (1-5) may be due to the significant effect caused by the study factors, including tea compost, which work to chelate most of the nutrients, especially the minor elements that work to stimulate the enzymes necessary for the formation of gibberellins and auxins, which then become centres of attraction. Nutrients that stimulate cambium cell division and increase their size [7]. This is reflected positively in most growth characteristics, such as the number of leaves, plant height, and number of branches [8], in addition to the concentration of N.P.K. elements within the organic matter, which is reflected positively in improving the properties of the soil, providing elements and raising its readiness for absorption by plants which positively affects various vital activities such as elongation and division of meristematic cells and the construction of proteins in addition to the formation of nucleic acids [9], and containing the substance organic matter contains a high percentage of major elements, such as nitrogen, which is primarily involved in building the chlorophyll molecule, in addition to its role in cell division and elongation, and this is directly reflected in an increase in the percentage of dry matter and the chlorophyll content of the leaves, as in Table (5) [10]. Organic matter also plays a major and effective role in increasing the activity of microorganisms, which in turn works to prepare the soil and plants with some necessary enzymes, including Protease and Phosphatase, which work to improve plant growth and increase root activity to absorb nutrients from the soil. It also works to form carbonic acid resulting from its decomposition, as it reduces the pH value of the soil and thus increases its microelements [11]. In addition, organic matter helps the root system grow by improving the chemical and physical properties of the soil, increasing the readiness of the necessary nutrients, and providing a suitable environment [12]. As a result, [13] explained that organic matter provides the agricultural medium with the nitrogen necessary for the metabolism of hormones and proteins, as it is primarily involved in their synthesis. It also works to raise the plant's content of amino acids, which is reflected positively in increased growth and diagonal division of root cells, which increases the accumulation of the substance. Dry inside the plant as in Table (4). The reason for the significant superiority in terms of treatment with natural plant extracts, especially when spraying with licorice root extract, may also be due to the extract containing Glycyrrhizin acid, which has an effective role in cell division and elongation, thus increasing the average root properties, which in turn is reflected in increased absorption rates, thus increasing the accumulation of Nutrients inside the plant, as well as containing the mevalonic, which behaves similar to gibberellin [14]. The reason for the superiority may be that the extract contains many nutritional elements that contribute to increasing vegetative growth, and it also contains sugars and stimulating compounds that regulate vegetative growth; the extract also affects enzymes for converting complex compounds into simpler compounds that the plant exploits in building new protein materials, necessary for its growth, and the plant cells may be able to absorb part of the extract's sugars and benefit from them in their vital activities, thus increasing the vegetative characteristics such as the height of the plant, the number of branches, and the number of vegetative branches, thus increasing its dry weight. As for the significant increase occurring in the chlorophyll content of the leaves due to the effect of the licorice extract, it may be because the extract contains terpene compounds, carbohydrates, and nutritional elements, the most important of which is Mg, which has a major role in building the chlorophyll molecule [15 ]. The results of the tables above show a significant reduction and decline in the values of the studied traits as a result of treatment with heavy elements. This may be due to the negative effect of these elements, as these elements increase the production of harmful (ROS) compounds for the plant, and to reduce their damage, the plant produces defensive secondary metabolic compounds that play an important role in protecting it from its dangers. It is also clear from the results that the interaction of the two factors of the study (extracts and tea compost) had a synergistic effect in increasing growth and this significant increase may be explained by the role of the plant in modifying the osmotic property of cells in a way that is proportional to the increase in the negative potential of the cell exposed to stress and its ability to survive. In conditions of soil contaminated with heavy elements due to the ability of its roots to withdraw and absorb these elements from the soil without causing significant damage to the construction and metabolic processes carried out by the plant [16,17]

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